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The effects of birth timing and ambient temperature on the hypothalamic-pituitary-adrenal axis in 3–4 month old rhesus monkeys

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Abstract

Birth timing, a relative measure of the timing of births within a season, has been shown to be related to the ways mothers and infant interact as well as to infant behavior and physiology. Although effects of birth timing on the hypothalamic-pituitary-adrenal (HPA) axis have previously been associated with variation in social relationships, these effects could also be related to seasonal variation in climate conditions when the birth season is long. The current study examines the effects of birth timing and ambient temperature on the activity and regulation of the HPA axis in 3–4 month old rhesus monkeys (N=338). Subjects were part of a biobehavioral assessment in which infants were separated from their mothers and relocated to a novel testing environment for a period of 25 hours. Four blood samples were collected and assayed for cortisol concentrations and reflected HPA response to 1) 2 hr maternal separation and relocation, 2) 7 hr maternal separation and relocation (sustained challenge), 3) dexamethasone suppression, and 4) ACTH challenge. Nonlinear mixed modeling was used to examine the independent effects of birth timing and temperature on HPA axis activity and regulation over the study period. Results indicated that birth timing and ambient temperature both had significant, but opposing effects on the cortisol response to sustained challenge. Chronic exposure to low ambient temperatures was associated with higher cortisol levels. After controlling for the effect of ambient temperature, birth timing was positively associated with cortisol such that late-born infants exhibited higher cortisol concentrations than did early-born infants. These results highlight the fact that climate conditions, even mild, subtropical conditions, can have potentially important influences on the activity and development of the HPA axis.

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Contributors

Dr. Vandelee conducted the study, analysis, and wrote the manuscript. Dr. Capitanio was her graduate advisor and provided funding and support throughout the study design, collection, analysis, and manuscript preparation phases of this project. Dr. Mendoza provided support for the analysis and interpretation of the data as well as assisted in manuscript preparation. Finally, Dr. Blozis provided significant assistance in the statistical analysis of these data. All authors contributed to and have approved the final manuscript.

Conflict of Interest

All authors declare that they have no conflicts of interest.

Keywords

Hypothalamic-pituitary-adrenal axis; Rhesus monkey; birth timing; ambient temperature

The hypothalamic-pituitary-adrenal (HPA) axis is a neuroendocrine system that is important for both the daily regulation of metabolism and the physiological response to stress. Activation of the axis begins when the hypothalamus releases corticotropin releasing hormone (CRH) which stimulates the anterior pituitary to release adrenocorticotrophic hormone (ACTH) into the blood stream. Upon reaching the cortex of the adrenal gland ACTH stimulates the release of glucocorticoids (cortisol or corticosterone). Cortisol release is regulated through a negative feedback loop involving tissues at multiple levels (e.g. hippocampus, hypothalamus, pituitary; Dallman et al., 1994; Herman et al., 1996; Herman et al., 2003). While cortisol is most often associated with regulation of metabolism and the stress response, cortisol can influence a wide variety of bodily functions and has been demonstrated to have effects on the cardiovascular system, fluid volume, immunity and inflammation, and reproductive physiology (Sapolsky et al., 2000). Due to the widespread influence of glucocorticoids on physiological systems, many researchers have sought to better understand the factors that influence the development of this system.

Maternal behavior has emerged as a strong organizer of the activity and regulation of the HPA axis (Francis and Meaney, 1999). For example, in rats, mothers that naturally exhibit lower levels of maternal care (less licking and grooming of pups) have offspring that show greater reactivity of the HPA axis including decreased negative feedback sensitivity and increased corticosterone responses to restraint stress (Francis and Meaney, 1999; Cameron et al., 2005). In addition to differences in hormone levels, maternal care has also been demonstrated to alter glucocorticoid receptor expression in the hippocampus (Francis et al., 1999), a brain region that is important in inhibiting HPA axis activity (Herman et al., 2003; Goursaud et al., 2006). Another example is illustrated by a recent study that demonstrated that egg incubation temperature (a factor regulated by maternal behavior) was associated with basal and stress-induced reactivity of the HPA axis in wood ducks (DuRant et al., 2010). Specifically, eggs that were incubated at a lower temperature (35.0°C versus 35.9°C) resulted in ducklings that exhibited higher basal and stress-induced corticosterone levels at 2 and 9 days post-hatching.

One factor that has been demonstrated to impact mother-infant interactions is birth timing, the relative timing of births within a social group. When a birth season is long, variation in birth timing may result in different social and environmental experiences for infants, which are likely to have consequences for infant development. Our prior work has investigated the impact of birth timing on the mother-infant relationship and infant behavioral and physiological development. Results of this research indicated that while the mother-infant relationship was more conflictual during weaning for late-born infants, during maternal breeding it was the earlyborn infants and their mothers that exhibited more conflict in the mother-infant relationship (Vandeleest and Capitanio, 2012; Vandeleest et al., 2012). In addition, when the mother-infant relationship was tense and conflictual during weaning, as was most likely for late-born infants, infants exhibited higher serum cortisol levels 3 weeks later (Vandeleest et al., 2012) and reduced antibody levels in response to a vaccination nearly 4 months later providing evidence that variation in the mother-infant relationship due to birth timing may have consequences for the functioning of the HPA axis and immunity.

Although we have demonstrated that birth timing can impact infant social relationships, the effects of birth timing on infant development may also reflect the influence of the non-social environment; for example, the seasonal variation in climate conditions across the birth

season. The birth season in the colony at the California National Primate Research Center (CNPRC) ranges from February to June; therefore infants born early versus late in this time period are likely to experience very different climate conditions early in development. Seasonal variation in glucocorticoid concentrations has been demonstrated in a variety of species (e.g. ground squirrel, Boswell et al., 1994; humans, Walker et al., 1997; red deer, Ingram et al., 1999; squirrel monkeys, Schiml et al., 1999). Although the triggers of seasonal changes in glucocorticoid release are unknown, there is evidence that temperature could be one contributing factor. One of the major effects of glucocorticoids is to regulate metabolic function, including increasing energy availability (e.g. increasing blood glucose) and thermogenesis. Studies in multiple mammalian species have reported that cortisol levels track the production of body heat in response to cold stress (Maickel et al., 1967; Olson et al., 1981; Werner and Vens-Cappell, 1985). In addition, experimentally reduced glucocorticoid levels led to an inability to thermoregulate when exposed to cold temperatures while glucocorticoid replacement restored the capacity for thermogenesis (Maickel et al., 1967; Werner and Vens-Cappell, 1985). Naturalistic studies in geese and sows have also reported a negative association between ambient temperature and glucocorticoid levels (Barb et al., 1991; Frigerio et al., 2004). Notably, a study in red deer indicated that fecal cortisol levels were predicted by minimum ambient temperatures but not maximum ambient temperature or precipitation levels (Huber et al., 2003). Because glucocorticoids, like cortisol, are so important for the maintenance of body temperature, differences in activity of the HPA axis between early and late-born infants may be due to climate or temperature-related factors.

The purpose of the current study was to examine the effects of birth timing on the activity of the HPA axis in a large sample of 3–4 month old rhesus monkey infants. A positive association between birth timing and cortisol would be consistent with effects we have previously described in a separate, smaller sample, which were mediated through the mother-infant relationship. A negative association, however, would be consistent with the cortisol's role in thermogenesis. To evaluate these two hypotheses we constructed a model in which the effects of birth timing and temperature on cortisol were examined to determine their independent effects on the activity and regulation of the HPA axis.

METHODS

Subjects and Housing

Subjects were 338 rhesus monkeys (50.9% male) housed at the California National Primate Research Center (CNPRC). Subjects included all animals that were born in the outdoor colony in 2008 and 2009 and had been participants in a BioBehavioral Assessment (BBA) program at CNPRC (see below). All animals were born and reared in one of nineteen 0.2 ha enclosures containing 100–175 animals with age and sex compositions similar to that in the wild. Cages contained multiple perches, climbing structures, and small shelters to provide protection from the rain and wind. Ample food was provided twice daily and water was available ad libitum. All experimental procedures were approved by the University of California-Davis Institutional Animal Care and Use Committee (IACUC) and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to reduce the number of animals used and minimize their suffering.

Birth timing

In the current sample infants were born between February 20, 2008 - June 3, 2008 (N=157), or January 16, 2009 - July 24, 2009 (N=181). Approximately 50% of animals were born in March and April each year. Birth timing was calculated within each cage and reflected the

number of days each subject was born after the first live infant was born in each cage (Mean = 40.5, Range: 0–142 days).

Ambient Temperature

The influence of seasonal temperatures on the HPA axis was determined by calculating the median minimum temperature between the date of birth and date of physiological assessment (during the BioBehavioral Assessment, see below) for each infant. While much of the research on temperature and the HPA axis has focused on proximate measures of temperature, our goal was to examine whether *chronic* exposure to low temperatures early in life would have an impact on the activity and regulation of the still developing HPA axis. Therefore we elected to use the median minimum temperature for approximately the first 3 months of life in our analyses. The minimum temperature was used because it has been previously suggested that minimum temperatures were more predictive of cortisol levels than maximum temperatures (Huber et al., 2003). The median minimum temperature ranged from 7.2–13.6°C (Mean = 10.9°C).

BioBehavioral Assessment

Measures of HPA axis function were obtained during a standardized 25-hour biobehavioral assessment when infants were 3–4 months (Mean = 3.5) of age. The methods are described elsewhere (Capitanio et al., 2006; Golub et al., 2009). Briefly, infants were separated from their mothers and relocated to a novel indoor testing environment where they were placed in temporary holding cages for a period of 25-hr. Water and food (standard monkey chow soaked in Tang) was available ad libitum and fruit was also provided. During this period a battery of tests was administered to assess the infant's behavioral and physiological reactivity. At the end of the 25-hr testing period infants were reunited with their mothers and returned to their natal cages. During the testing period four blood samples were drawn from each monkey via femoral venipuncture following brief manual restraint. The first sample was drawn at 1100h (1 ml), approximately 2 hours after maternal separation and relocation to the testing environment, and presumably reflected the immediate cortisol response to these events. The second sample was drawn at approximately 1600h on the first day of testing (0.5 ml). The normal diurnal cycle of cortisol secretion results in a decrease in cortisol levels in the afternoon, thus this sample may reflect the HPA axis response to sustained challenge. After the second sample was drawn infants were given an intramuscular injection of 500 µg/kg dexamethasone, a synthetic glucocorticoid (American Regent Laboratories, Inc., Shirley, NY). The third sample (0.5 ml) was drawn at 0830h the next day and reflected the potential suppression of endogenous cortisol release by dexamethasone. After the third sample was drawn infants were injected with 2.5 IU ACTH (Organon, Inc. West Orange, NJ) intramuscularly and a fourth blood sample (0.5ml) was obtained 30 minutes later. This fourth sample reflected the ability of the adrenal cortex to respond to ACTH stimulation. Once collected, blood samples were immediately transferred to EDTA tubes and centrifuged at 4°C at 1277 RCF for 10 min, plasma was then extracted and stored at –80°C until assay. All experimental procedures were approved by the University of California-Davis Institutional Animal Care and Use Committee (IACUC).

Cortisol Assay

Prior to assay, samples were diluted 1:6 in PBS gel buffer. Plasma concentrations of cortisol were estimated in duplicate using commercial radioimmunoassay kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Assay procedures were modified with the addition of 0.5 and 2.5 µg/dL concentrations of standards along with the provided range of 1.0 to 50 µg/dL. This assay has been continuously in use since 1989 for assay of plasma cortisol concentrations in nonhuman primates, including rhesus (Capitanio et al., 1996; Capitanio et al., 1998; Capitanio et al., 2005; Goursaud et al., 2006; Vandeleeest et al., 2012). Validation

of the assay used ANCOVA to compare the slopes of serial dilutions from 2 rhesus monkeys to kit standards which demonstrated parallelism ($F(1,13) = .006, p=.941$). Intra- and inter-assay coefficients of variation were 5.18% and 8.30%, respectively. Assay sensitivity has been determined to be 0.26069 $\mu\text{g/dL}$.

Data analysis

The relationships between birth timing, temperature, and plasma cortisol at each time point were initially explored using bivariate correlations. Data were then analyzed using nonlinear mixed-effects models (estimated by PROC NLMIXED, SAS version 9.0) with repeated cortisol measurements as time-varying outcome measures (level 1) and birth timing and median minimum temperature as individual-level variables (level 2). Infant sex and age at BBA testing/blood sampling were also included at the second level of the model as covariates. Effects of cohort (2008 or 2009) were examined and were non-significant. Mixed modeling techniques are more flexible than conventional repeated-measures or linear regression techniques as they simultaneously allow for flexible specification of the variances and covariances between repeated measures, examination of the influence of continuous predictors on repeated measures outcomes, and variation in the number and spacing of time points within individuals (Raudenbush and Bryk, 2002). Due to the nonlinear form of change in the outcome measures over the 4 time points, a multiphase model was fitted to the data (Cudeck and Klebe, 2002). The first phase of the model represented the natural reactivity of the HPA axis to the testing situation and assumed linear change from sample 1 to 2, where at sample 2, cortisol secretion was expected to change course (see Figure 1); the second phase of the model represented the response of the HPA axis to pharmacological challenge and assumed quadratic change from sample 2 to sample 4. Time was centered at the second blood sample, the transition point that joined the linear and quadratic phases of the model. Because the distribution of cortisol was highly kurtotic, the natural log of cortisol was used for the repeated measures analyses. Age and temperature were centered at their respective minimum values (Age = 90.0 days, Temperature = 7.2°C). Therefore the intercept (i.e., cortisol level at Sample 2) reflects the natural log of afternoon cortisol values for 90 day old females that were born on the first day of the birth season with a median ambient temperature of 7.2°C.

The first step of the analysis consisted of constructing a multiphase model and evaluating the addition of random effects to the coefficients of the growth model to determine if there were significant individual differences in the change in cortisol over the testing period. Next, covariates (sex and age at BBA testing), birth timing, and temperature were added to the model to determine if they predicted 1) the intercept, 2) the slope of the linear phase (phase 1) of the model, 3) the instantaneous rate of change between the first and second phases (i.e., at the transition point), and 4) the quadratic effect of the second phase. Conceptually, these steps examine the effects of predictors of 1) afternoon cortisol levels (sample 2), 2) change in cortisol from initial response to separation and relocation to the afternoon sample (sample 1 to sample 2), 3) initial response to dexamethasone (initial rate of change after sample 2), and 4) response to ACTH following dexamethasone (quadratic change from sample 2 to sample 4). Evaluation of the effects of the predictors was done by fitting a sequence of models. First, tests were performed of the effects of all 4 predictors on the intercept of the model. During model building, effects that were not statistically significant at the .10 level using two-tailed tests were removed and the model refitted. In the final model, however, a more stringent a criterion of .05 was used to determine the significance of parameter estimates. Similar steps were taken to evaluate the effects of the predictors on the linear slope of the first phase, the instantaneous change rate between phases, and the quadratic effect of the second phase. All pairwise interactions were also evaluated for each segment of the model; all were non-significant and were not included in the final model. Differences in

overall fit between models was determined by examining the change in the -2 log-likelihood between nested models and evaluated as a chi square statistic with degrees of freedom equal to the difference in the number of parameters used to define each model. The proportion of variance explained by the level 2 predictors, a measure of effect size, was approximated by calculating the percent reduction in the variance of the random effect between a model containing the level 2 predictors and a “base” model with the predictors absent (Raudenbush and Bryk, 2002).

RESULTS

Birth timing and temperature were negatively correlated with cortisol concentrations at all four time points. The correlations, however, were small and only significant for birth timing at samples 2 and 4 whereas the correlations were small but significant for temperature at all four time points (Table 1). Because birth timing and temperature were correlated with each other ($r = 0.702$, $p < 0.001$), both variables were included in the subsequent analysis to determine their independent effects on cortisol levels.

Formation of a nonlinear model

Change in cortisol over the testing period was described by a model that assumed an initial linear increase in cortisol across the first day of testing followed by a decrease in cortisol in response to dexamethasone administration and an increase in cortisol after ACTH was administered (Figure 1). The first model fitted included a random intercept to address the dependencies of scores within animals. Additional random effects were then added to the model to test whether there was individual variation in the remaining coefficients that described change in cortisol responses. Based on a multiphase model that included a random intercept, the results suggested that there was on average a significant increase in cortisol from samples 1 to 2 (Table 2, Line 6). The results also suggested that at the time of transition between phases there was a significant mean decrease in cortisol levels, indicating that cortisol levels decreased after dexamethasone was administered (Table 2, Line 7). Finally, the quadratic effect (rate of acceleration) of the second phase of the model was statistically significant indicating that on average the rate of change in cortisol levels accelerated from sample 2 to sample 4 (Table 2, Line 10). On average, cortisol levels were $85.63 \mu\text{g/dL}$ at sample 2. Significant variation in the random intercept, however, as determined by fitting a model that assumed a fixed intercept ($X^2 = 648.5$, $df = 1$, $p < 0.001$), indicated individual differences in cortisol levels at sample 2 (Table 2, Line 12). Maximum likelihood estimates with standard errors are reported in Table 2.

Predicting variation in cortisol levels at the intercept (Sample 2)

Significant predictors of afternoon cortisol levels (Sample 2) included covariates (age and sex) as well as ambient temperature and birth timing. The addition of these four factors significantly improved model fit ($X^2 = 52.4$, $df = 4$, $p < 0.05$). The estimated effects indicated that infants that experienced lower minimum temperatures during their first 3–4 months of life had higher cortisol levels at Sample 2, explaining approximately 5.5% of the individual differences in afternoon cortisol levels (Table 2, Line 5; Figure 2). In contrast, after controlling for the effect of ambient temperature, the effect of birth timing indicated that late-born infants were more likely to have higher afternoon cortisol levels, explaining approximately 1.8% of the variance in cortisol levels (Table 2, Line 4). Examination of the covariates indicated that animals that were older at BBA testing were more likely to exhibit higher afternoon cortisol levels which explained approximately 2.5% of the variance in afternoon cortisol levels (Table 2, Line 3). Finally, the effect of sex indicated that males had lower afternoon plasma cortisol concentrations, explaining approximately 3.3% of the variance in afternoon cortisol levels (Table 2, Line 2; Figure 1).

Predicting the rate of change in cortisol from samples 1 to 2 (Phase 1)

There were no significant predictors of the rate of change in cortisol levels (represented by the linear slope of the first model phase) from the initial response to separation and relocation to the afternoon sample (sample 1 to sample 2).

Predicting the immediate response to dexamethasone (initial rate of change in Phase 2)

Ambient temperature and sex predicted the initial decline in cortisol values in response to administration of dexamethasone, an indicator of negative feedback efficacy, and significantly improved overall model fit ($X^2 = 18.5$, $df = 2$, $p < 0.05$). Lower minimum temperatures experienced in the first 3 months of life were associated with steeper initial declines in cortisol in response to dexamethasone (Table 2, Line 9; Figure 2). Similarly, males exhibited steeper initial declines in cortisol in response to dexamethasone (Table 2, Line 8; Figure 1). Although these effects were statistically significant they resulted in small differences in the initial rate of change. When converted back into $\mu\text{g/dL}$ of cortisol, the mean initial rate of change between samples 2 and 3 was 1.82 for females versus 2.09 for males. In addition, 1 degree increase in temperature was associated with only a 0.01 $\mu\text{g/dL}$ difference in the initial rate of change, a statistical difference that is unlikely to be biologically meaningful.

Predicting the rate of acceleration in cortisol levels in Phase 2

Only infant sex predicted the quadratic effect of the second phase of the model (Model fit improved: $X^2 = 6.0$, $df = 1$, $p < 0.05$). Males exhibited a faster rate of acceleration between the second and fourth blood samples (see Figure 1), but this effect was small (Table 2, Line 11). After conversion back into $\mu\text{g/dL}$ of cortisol, acceleration from sample 2 to 4 was 1.42 for females and 1.50 for males, a difference of 0.08.

DISCUSSION

The current study explored the effects of birth timing and seasonal temperature variation on the HPA axis in infant rhesus monkeys. In general, we found the same pattern of cortisol secretion across the BBA testing period as was previously reported for infants reared in large, naturalistic social groups (Capitanio et al., 2005). Specifically, cortisol showed a small increase across the first day of testing (sample 1 to sample 2), dexamethasone resulted in a significant decrease in cortisol, and the administration of ACTH resulted in an increase in cortisol. The present results also indicated that infants that experienced lower minimum temperatures in their first 3 months of life had higher plasma cortisol levels, suggesting a potentially important influence of seasonal temperature conditions on the activity of the HPA axis. In addition, while bivariate correlations indicated a negative relationship between birth timing and infant cortisol levels, multilevel modeling indicated that when the effects of seasonal temperatures variation were controlled, birth timing had a positive association with infant plasma cortisol levels.

A variety of studies have found that activity and regulation of the HPA axis is influenced by early rearing experience (Meyer et al., 1975; Shannon et al, 1998; Capitanio et al, 2005). In our BBA program, nursery-reared infants exhibited lower average cortisol levels across the testing period perhaps indicating that early rearing may alter the “set-point” of the HPA axis (Capitanio et al., 2005). Early rearing also likely impacts the regulation of the HPA axis; while cortisol secretion is expected to decline across the day, animals reared by their mothers in large enclosures consistently showed an increase in cortisol secretion across the first day of testing (change from sample 1 to 2). In contrast, nursery reared animals exhibited either no change or a decline in cortisol despite the ongoing challenge of the testing situation (Capitanio et al., 2005). In addition, while the expected dexamethasone suppression of

endogenous cortisol secretion was consistently evident among animals reared by their mothers in large social groups, the effects were mixed for animals reared by their mothers indoors or with a peer in a nursery (Capitanio et al., 2005). While these earlier studies demonstrated that gross differences in the early rearing environment impact HPA axis activity and regulation, the current study expands those findings to explore two more subtle predictors of individual variation in activity and regulation of the HPA axis among normally reared animals, birth timing and temperature.

Ambient Temperature

The present results indicate a role for seasonal changes in ambient temperature on HPA axis development, specifically low temperatures. Consistent with previous studies (Barb et al., 1991; Huber et al., 2003; Frigerio et al., 2004), the current study found that lower minimum temperatures were associated with higher cortisol levels (Barb et al., 1991; Huber et al., 2003; Frigerio et al., 2004), accounting for approximately 5.5% of the individual differences in afternoon cortisol levels. Notably, this relationship was found despite our use of a measure that reflected general temperature conditions over an extended period of time and all blood samples were collected in a thermally regulated environment. This could indicate that chronic exposure to low temperatures may have a long lasting influence on HPA axis reactivity.

The higher cortisol levels after exposure to lower temperatures may result from cortisol's role in thermogenesis (Maickel et al., 1967; Werner and Vens-Cappell, 1985). We believe that the effect of temperature on cortisol concentrations is likely to be mainly a reflection of differential metabolic demands induced by climate conditions. It is important to note that while the effect of temperature was small, the climate at the CNPRC is quite mild (range of median temperatures was only 6.4°C). It is possible that in more extreme or variable climates (e.g., for species at higher altitudes or latitudes) the effect of ambient temperatures on HPA axis activity may be even stronger. Although we demonstrated that low ambient temperature influences HPA axis activity, there are many other seasonal cues that were not examined in the current study that could also impact the activity of the HPA axis. It is possible that these other cues, including variation in daylight, precipitation, and humidity, may also contribute to seasonal changes in the activity of the HPA axis. Finally, although not measured here it is also important to note that environmental pollutants and contaminants may also impact cortisol levels (Bechshøft et al., 2012).

Birth Timing

When a female conceives, and consequently, when she gives birth is likely influenced by a wide range of factors including the availability of food resources, outcomes of previous pregnancies, and social rank (Tanaka et al., 1970; Fedigan and Rose, 1995; Emery Thompson et al., 2007). Our exploration of birth timing has focused not on what causes variability in birth timing, but on the consequences of variation in birth timing for infant development. The current study provides evidence that birth timing has an influence on the functioning of the HPA axis, and that "birth timing" may reflect multiple factors that can impact infant physiology. While initial bivariate correlations indicated a negative relationship between birth timing and cortisol levels, further analysis indicated that this effect was mainly driven by seasonal variation in ambient temperature. When ambient temperature was controlled, birth timing had a positive relationship with cortisol. This positive association is consistent with our previous findings (from a separate, smaller sample) that late-born infants had more tense relationships with their mothers during weaning and that this was associated with higher cortisol at 6 months of age (Vandeleest et al., 2012).

Other factors

Covariates in the model also indicate significant effects for infant sex on afternoon cortisol levels and cortisol in response to pharmacological challenges, and an effect of infant age on afternoon cortisol levels. We found that males exhibited lower afternoon cortisol concentrations which is consistent with some previously reported effects of sex (Scallet et al., 1981; Clarke, 1993; Capitanio et al., 2005), although other studies report inconsistent effects or no effect of sex on HPA axis activity (Kudielka & Kirschbaum, 2005; Koch et al., 2012). Sex explained approximately 3.4% of the variance between individuals which is similar to the size of the sex effect that has been previously reported for animals from this colony (Capitanio et al., 2005). Unlike previous reports from our lab in which females were suggested to display greater dexamethasone suppression, the current study found that males exhibited greater dexamethasone suppression. Caution is required in interpreting the effects of sex in the current study, however, because the effects of sex on the cortisol responses to pharmacological challenge (the second phase of the model) explained less than 1% of the variance in the model. Although these effects are statistically significant, due to the small effect sizes it is unclear whether these sex differences are physiologically meaningful. The present results also indicate a small effect of age on cortisol levels. In general, infant primates have been shown to exhibit higher cortisol levels than juveniles or adults (personal observation; Kiess et al., 1995; Pryce et al., 2002). Our findings suggest that within the infancy period, age is positively correlated with cortisol levels, possibly due to cortisol's association with greater body mass (Kiess et al., 1995).

Overall, the current study provides evidence for the effects of birth timing on the HPA axis, but suggests that birth timing may be a complex measure that reflects multiple aspects of the early environment. The effect of birth timing reported in the current study is consistent with previously reported effects of birth timing on the HPA axis in which the effects of birth timing were suggested to be mediated through social relationships (i.e. the mother-infant relationship; Vandeleest et al., 2012). In addition, the current study suggests that when the birth season in a species is long, climate variations may also impact infant physiological systems. Altogether these data indicate that there are multiple factors that contribute to the effects of birth timing on infant development.

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Figure 1. Mean Cortisol Values by Sex

Mean cortisol values across the BBA testing period for males and females (Mean $\pm$ SE).

Raw data are presented.

Figure 2. Mean Cortisol Values for Temperature Extremes

Mean cortisol values across the BBA testing period for animals experiencing the highest median temperatures (12.5–13.6°C, N = 61) and lowest median temperatures (7.2–8.3°C, N = 59). Raw data are presented (Mean ± SE).

Table 1

Correlations between repeated measures of cortisol and birth timing and temperature

	Cortisol			
	Sample 1	Sample 2	Sample 3	Sample 4
Birth timing	-0.102 ⁺	-0.154 [*]	-0.100 ⁺	-0.183 [*]
Temperature	-0.196 [*]	-0.229 [*]	-0.210 [*]	-0.316 [*]

⁺
p<0.1,^{*}
p<0.05

Table 2

Parameter Estimates for the Final Mixed Model

Line	Predictor	MLE	SE	t
1	Intercept (Cortisol level at Sample 2)	4.45	0.037	120.35 *
2	Sex (Female=0, Male=1)	-0.095	0.028	-3.37 *
3	Age	0.0046	0.0017	2.71 *
4	Birth timing	0.0016	0.00069	2.28 *
5	Temperature	-0.029	0.0069	-4.17 *
6	Linear Slope (Phase 1)	0.059	0.014	4.22 *
7	Slope (Phase 2)	-0.60	0.037	-16.35 *
8	Sex	-0.14	0.046	-3.06 *
9	Temperature	-0.0059	0.0019	-3.12 *
10	Quadratic effect (Phase 2)	0.35	0.017	20.75 *
11	Sex	0.057	0.023	2.45 *
Variance Components				
12	Random Intercept	0.049	0.0044	22.54
13	Time-specific error	0.034	0.0015	11.08

MLE is maximum likelihood estimate. SE is the estimated standard error. *t* is the ratio of MLE/SE;

* *t*-values above 1.96 or below -1.96 are statistically significant at the .05 level.